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Fim-1, *Fim-2*/c-*fms*, and *Fim-3*, Three Common Integration Sites of Friend Murine Leukemia Virus in Myeloblastic Leukemias, Map to Mouse Chromosomes 13, 18, and 3, Respectively

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Three common proviral integration sites, *Fim-1*, *Fim-2*/c-*fms*, and *Fim-3*, have been described in mouse myeloid leukemias induced by the Friend murine leukemia virus. The nature and function of *Fim-1* and *Fim-3* are still unknown since no transcript from these loci has been detected so far. To identify these two loci, we undertook their chromosomal localization using restriction fragment length polymorphism detected between C57BL/6 mice and the wild-derived inbred strain of *Mus spretus*. Using interspecific backcross analysis, we mapped *Fim-1* to mouse chromosome 13 and *Fim-3* to mouse chromosome 3. Interestingly, *Fim-3* is tightly linked to *Evi-1*, another common integration site of ecotropic virus involved in another model of mouse myeloid leukemogenesis. *Fim-2* spans the 5′ end of the c-*fms* gene, which encodes for the macrophage-colony-stimulating factor receptor. We located the c-*fms* gene on the D band of chromosome 18 by in situ hybridization.

Nonacute leukemia viruses induce cellular transformation mainly by an insertional mutagenesis mechanism. Thus, proto-oncogenes such as c-*erbB* (7), c-*myc* (5, 16, 29), and c-*myb* (28, 37) are frequently activated by retroviral integration either by downstream promotion or by an enhancer effect of the proviral long terminal repeat. In addition, numerous putative proto-oncogenes are activated by retroviral integration. They were recently discovered by the "transposon tagging" strategy: the newly acquired proviruses in tumor cells are used as probes to identify cellular loci implicated in the transformation process. By cloning virus/host cellular junction fragments, unique cellular sequences are identified and used to screen other tumors. By this approach, common proviral integration sites were described in different species and in different virus-induced tumor systems such as *Int-1* (31), *Int-2* (33), *Int-3* (8), and *Int-41* (9) for the mouse mammary tumor virus-induced mammary carcinomas; *Spi-1* for spleen focus-forming virus-induced erythroleukemias (25); and *Pim-1* (6), *Fis-1* (39), *Gin-1* (47), *Mlvi-1* (44), *Mlvi-2* and *Mlvi-3* (45), *Mis-1*/*Pvt-1* (12, 21), *Dsi-1* (46), *Evi-1* (27), and *Evi-2* (4) for murine leukemia virus (MuLV)-induced mouse or rat leukemias.

We have previously described three common proviral integration sites of the Friend MuLV (F-MuLV) in mouse myeloblastic leukemias (2, 41). These three regions, *Fim-1*, *Fim-2*, and *Fim-3*, are involved in 3, 26, and 26%, respectively, of the leukemias tested. *Fim-2* spans the 5′ end of the c-*fms* proto-oncogene, and proviral insertion in this region leads to the overexpression of a normal-sized c-*fms* mRNA (10). Since c-*fms* codes for the macrophage-colony-stimulating factor (M-CSF) receptor, we can infer that overexpression of this receptor may play a role in myeloblastic leukemogenesis. *Fim-1* and *Fim-3* are well conserved throughout evolution, but their nature and function are still under investigation. We undertook their chromosomal mapping to verify that these two regions were different from proto-oncogenes, growth factor genes, or growth factor receptor genes and from previously described common integration sites. This was done using the restriction fragment length polymorphisms (RFLPs) detected in interspecific mouse backcrosses. *Mus spretus* is very useful for genetic analysis since it is evolutionary divergent from the classical inbred laboratory strains and polymorphism is detected at many loci between the two species of mice (14, 35). In addition, the chromosomal location of the mouse c-*fms* gene was determined by the in situ hybridization technique.

We found that *Fim-1* maps to chromosome 13, *Fim-3* to chromosome 3, and *Fim-2*/c-*fms* to chromosome 18. The genetic linkage between *Fim-3* and *Evi-1*, a common site of ecotropic viral integration in AKXD murine myeloid tumors (27), is also reported.

MATERIALS AND METHODS

Mice. C57BL/6, BALB/c, WMP/Pas, and SPE/Pas (*Mus spretus*) mice and the interspecific backcross progeny (C57BL/6 × SPE)F_1 × C57BL/6 or (BALB/c × SPE)F_1 × BALB/c were all raised at the Institut Pasteur, Paris, France.

Male backcross animals used in this study were also characterized by F. Bonhomme and colleagues (Centre National de la Recherche Scientifique UA327, Montpellier, France) for the segregation of 15 biochemical markers already localized on the mouse genetic map.

DNA isolation and Southern blot analysis. High-molecular-weight DNA was extracted from frozen spleen as previously described (48). DNAs (5 μg) were digested with appropriate restriction enzymes under reaction conditions recommended by the manufacturers. Agarose gel electrophoresis, Southern

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TABLE 1. Probes used in interspecific mouse backcross studies

Probe	Gene or locus	Chromosome	Reference(s)
pUC1.0HP	*Evi-1*	3	26, 27
$MLC1_A$	Myosin light-chain gene	11	35
pGH5'19	GM-CSF	11	11
VTγ5	Variable region of the γ chain of the T-cell receptor gene	13	19, 32, 40
CTLA-3	Serine esterase gene	13	3
4/12	Integration site of hepatitis B virus in transgenic mice	13	15
PL5	Anonymous probe	13	22
MPB	Myelin basic protein gene	18	24, 30, 38

blot transfer, and hybridization were performed as previously described (42).

Chromosome spread preparation. In situ hybridization experiments were carried out using metaphase spreads from a male mouse of the WMP/Pas inbred strain, in which all autosomes except 19 are involved in Robertsonian translocations. Concanavalin A-stimulated lymphocytes were cultured at 37°C for 72 h, with 5-bromodeoxyuridine added for the final 6 h of culture (60 μg/ml of medium) to ensure a good-quality chromosomal R-banding.

Probe preparation and in situ hybridization. The *Fim-2* 17(1)-BX11 probe was tritium labeled by nick-translation to a specific activity of 8.8×10^7 dpm/μg. The radiolabeled probe was hybridized to metaphase spreads at a final concentration of 100 ng/ml of hybridization solution as previously described (23).

Autoradiography, staining, and banding. After coating with nuclear track emulsion (Kodak NTB_2), the slides were exposed for 8 days at 4°C and then developed. To avoid any slipping of silver grains during the banding procedure, chromosome spreads were first stained with buffered Giemsa solution and metaphases were photographed. R-banding was then performed by the fluorochrome-photolysis-Giemsa method, and metaphases were rephotographed before analysis.

Probes. Probes corresponding to the three *Fim* regions used for hybridization were as follows: for *Fim-1*, a 0.9-kilobase (kb) *Eco*RI-*Hin*dIII fragment (51-EH09); for *Fim-2*, a 0.4-kb *Pvu*II-*Bst*EII fragment (341-PB04); and for *Fim-3*, a 2.0-kb *Pvu*II fragment (206-PP20). In addition, the *Fim-2* 17(1)-BX11 plasmid, containing a 1.1-kb *Bam*HI-*Xba*I fragment inserted into pUC13, was used for in situ hybridization. All these probes contain unique cellular sequences and have been previously described (2, 41; S. Fichelson, B. Sola, F. Dreyfus, D. Bordereaux, S. Gisselbrecht, and P. Tambourin, Leukemia, in press).

Probes used as markers for chromosomal mapping are summarized in Table 1.

RESULTS

To identify RFLPs at the *Fim-1*, *Fim-2*/c-*fms*, and *Fim-3* loci, we analyzed DNAs of four inbred laboratory mouse strains (C57BL/6, BALB/c, DBA/2, and ICFW mice), using seven restriction enzymes with probes derived from the three *Fim* regions. Since no polymorphism was detected, we examined DNA of an inbred strain of *Mus spretus* (SPE/Pas) with the same probes. RFLPs were found at all three loci examined. The *Fim-1* 51-EH09 probe detected two 5.8- and 8.4-kb *Hin*dIII fragments in C57BL/6 DNA and a unique 3.5-kb *Hin*dIII fragment in SPE DNA. The *Fim-2* 341-PB04 probe detected a 25-kb *Eco*RI fragment in C57BL/6 DNA and a 17-kb *Eco*RI fragment in SPE DNA. The 206-PP20 *Fim-3* probe detected a 7-kb *Bam*HI fragment in C57BL/6 DNA and a 12-kb *Bam*HI fragment in SPE DNA. We therefore hybridized DNAs from interspecific backcross mice with these probes to look for cosegregation with known genetic markers.

TABLE 2. Segregation of *Fim-1* and chromosome 13 markers in interspecific backcrosses[a]

Marker	4/12	PL5	*Fim-1*	Vtγ5	CTLA-3
4/12	—	1/49 (2.0 ± 2.0)	10/49 (20.4 ± 5.7)	12/48 (25.0 ± 6.2)	16/48 (33.3 ± 6.8)
PL5		—	11/75 (14.6 ± 4.0)	12/48 (25.0 ± 6.2)	30/74 (40.5 ± 5.7)
Fim-1			—	7/48 (14.5 ± 5.0)	35/74 (47.3 ± 5.8)
VTγ5				—	16/47 (34.0 ± 6.9)
CTLA-3					—

[a] Numbers refer to the number of recombinants per total number of animals analyzed. Parentheses indicate percent recombination ± standard deviation.

Chromosomal localization of *Fim-1* and *Fim-3*. Studies of interspecific backcrosses indicated that *Fim-1* was located on chromosome 13. The chromosome 13 markers used were a VTγ5 probe isolated from the variable region of the γ chain of the T-cell receptor gene (40); PL5, an anonymous probe (22); 4/12, a probe that corresponds to a flanking region of an integrated hepatitis B virus into a transgenic strain of mouse (15); and a CTLA-3 probe cloned from the serine esterase gene (3) (Table 2). No evidence of linkage was observed between CTLA-3 and any other chromosome 13 markers. This result is in agreement with in situ hybridization experiments which have located CTLA-3 on the distal part of chromosome 13 (M.-G. Mattéi, unpublished results).

In contrast, genetic linkage was observed between all other markers. Only one recombinant was detected between PL5 and 4/12, indicating that these two loci are separated by 2.0 centimorgans (cM) (95% confidence limit). The genetic distance between *Fim-1* and PL5 (14.6 ± 4.0 cM) and *Fim-1* and VTγ5 (14.5 ± 5.0 cM) on one hand, and PL5 and VTγ5 (25.0 ± 6.2 cM) on the other hand, indicates that *Fim-1* is localized approximately at the same distance between these two loci. The γ chain of the murine T-cell receptor is known to map near the beige coat color locus on the proximal part of chromosome 13 (19, 32). This would suggest a gene order of centromere–γ chain–*Fim-1*–PL5–4/12-CTLA-3 (see Table 2).

No cosegregation of *Fim-1* and *Fim-3* was observed. Instead, *Fim-3* was linked to chromosome 3 markers. The segregation of *Fim-3* with chromosome 3 markers (Adh-1, Amy, Hao-2, Pk-1) is presented and analyzed in Table 3. These results indicate that *Fim-3* is located at 37.3 ± 5.6 cM from Adh-1, 33.8 ± 5.5 cM from Amy, 29.3 ± 5.3 cM from Hao-2, and 32.6 ± 6.7 cM from Pk-1.

A common site of integration of ecotropic MuLV in AKXD myeloid tumors (*Evi-1*) has been recently described (27) and found to be localized on the proximal part of mouse chromosome 3 (26). To determine the genetic linkage between *Evi-1* and *Fim-3*, *Evi-1* segregation was studied by Southern blot analysis using a *Taq*I RFLP which allows differentiation between C57BL/6 and *M. spretus* DNAs (26). Among 90 backcross mice, no recombinant was detected between *Fim-3* and *Evi-1*, indicating that these two loci are tightly linked (<2 cM at the 95% limit).

TABLE 3. Segregation of *Fim-3* and chromosome 3 markers in interspecific backcrosses[a]

Marker	*Fim-3*	*Evi-1*	Adh-1	Amy	Hao-2	PK-1
Fim-3	—	0/90	28/75 (37.3 ± 5.6)	25/74 (33.8 ± 5.5)	22/75 (29.3 ± 5.3)	16/49 (32.6 ± 6.7)
Adh-1			—	13/74 (17.6 ± 4.4)	18/75 (24.0 ± 4.9)	17/49 (34.7 ± 6.8)
Amy				—	7/74 (9.4 ± 3.4)	8/49 (16.3 ± 5.3)
Hao-2					—	5/49 (10.2 ± 4.3)
PK-1						—

[a] Numbers refer to the number of recombinants per total number of animals analyzed. Parentheses indicate percent recombination ± standard deviation. These data suggest a gene order of centromere–*Fim-3*/*Evi-1*–PK-1–Hao-2–Amy–Adh-1.

Localization of *Fim-2*/c-*fms* on chromosome 18 by in situ hybridization. No clear genetic linkage between *Fim-2*/c-*fms* and any chromosome marker could be found by using interspecific backcross analysis. In particular, no cosegregation was observed with *Fim-1*, *Fim-3*, and 48 chromosome markers. The human c-*FMS* gene has been localized on human chromosome 5 at band 5q34 (13, 36) and is linked to the IL3 and GM-CSF genes localized at band 5q23-31 (20, 34). Murine IL3 and GM-CSF genes have been found to be linked on mouse chromosome 11 by both interspecific backcross analysis and pulsed-field gel electrophoresis (1). To determine whether c-*fms* is also on mouse chromosome 11, we used *Bam*HI RFLPs for the GM-CSF locus. Two other chromosome 11 markers were used, the biochemical Esterase-3 marker and the $MLC1_A$ probe, corresponding to the cardiac myosin light-chain gene (Myla) (35). Results of segregation were inconsistent with chromosome 11 linkage (data not shown). Therefore, *Fim-2*/c-*fms* location was studied by in situ hybridization on mouse metaphase preparations. In the 100 metaphase cells examined, 172 silver grains were associated with chromosomes and 35 of these (20%) were located on chromosome 18. The distribution of grains on this chromosome was not random: 85% of them mapped to the (C-D-E) region of chromosome 18, with a maximum in the D band (Fig. 1). These data allowed us to map the *Fim-2*/c-*fms* gene to the D band of chromosome 18. This result is consistent with the percentage of recombination obtained with the MBP chromosome 18 marker (33.3 ± 6.0 cM) used in interspecific backcross analysis, since the MBP gene is located at the distal part of mouse chromosome 18 (38).

DISCUSSION

We have cloned three common integration sites of F-MuLV in murine myeloblastic leukemias (2, 41). One of these sites, *Fim-2*, spans the 5′ end of the murine c-*fms* gene (10). The nature of the genes activated by insertion in the *Fim-1* and *Fim-3* regions is still unknown since no mRNA corresponding to these integration regions has so far been detected. We knew from mRNA analysis that integration of proviruses in the *Fim-1* or in the *Fim-3* region did not result in high expression of c-*fms* mRNA. Moreover, no correlation was found with the expression either of other oncogenes such as c-Ha-*ras*, c-Ki-*ras*, c-*myc*, c-*myb*, p53, c-*fes*, c-*abl*, c-*kit*, or *Pim-1* or of growth factor genes such as IL3, G-CSF, M-CSF, GM-CSF, or Epo (data not shown). We have shown in this study that the three *Fim* regions are located on different mouse chromosomes. This eliminates the possibility that *Fim-1* and *Fim-3* represent different parts of the same locus.

By examining restriction enzyme polymorphism between *M. spretus* and BALB/c or C57BL/6 mice, we were able to map *Fim-1* and *Fim-3* to mouse chromosomes 13 and 3, respectively. *Fim-1* was located at 14.5 ± 5.0 cM of the variable region of the γ chain of the T-cell receptor gene and at the same distance from the PL5 marker. An insertion locus of the hepatitis B genome in transgenic mice (4/12 sequences) has recently been mapped to mouse chromosome 13 (15). This insertional locus is at 2.0 ± 2.0 cM of the PL5 marker and is genetically linked to the *Fim-1* integration region. Interestingly, no oncogene, growth factor gene, or MuLV integration site has yet been assigned to chromosome 13.

Data from interspecific backcross analysis placed *Fim-3* on chromosome 3 but at some distance from all the markers used. A new integration site of ecotropic MuLV in spontaneous myeloid tumors of AKXD mice, called *Evi-1*, has recently been mapped on the proximal part of chromosome 3 (26, 27). Our genetic analysis showed that *Fim-3* and *Evi-1* were separated by less than 2 cM, since no recombinant between the two loci was detected among 90 animals tested. The published maps of *Evi-1* and *Fim-3* are different, and therefore they do not represent identical regions of the same locus. Proviruses inserted in the *Fim-3* region are not clustered but rather scattered over at least 17 kb. In fact, the extreme limits of the *Fim-3* integration region have not been determined. In contrast, proviral integration in the *Evi-1* region is limited to less than 0.6 kb (27). Furthermore, we analyzed 42 F-MuLV-induced myeloid leukemias, previously tested for *Fim-3* rearrangements, with the *Evi-1* pUC1.0HP probe. Three tumors were rearranged with the *Evi-1* probe (data not shown). None of these three tumors had proviruses inserted into the *Fim-3* region. Therefore the mouse chromosomal domains hybridizing with *Fim-3* and *Evi-1* probes do not overlap. *Fim-3* and *Evi-1* could be different regions of the same locus or could represent different genetic loci both involved in myeloid leukemogenesis. At least four different regions of MuLV integration involved in lymphoid leukemogenesis have been mapped on mouse chromosome 15: *Mlvi-1*, *Mlvi-2*, *Mis-1*/*Pvt-1*, and c-*myc*. *Mlvi-1* and *Mlvi-2*, which are localized on the same mouse chromosome, are located on different rat chromosomes and therefore correspond to different genetic loci (43). Such an approach could help in determining whether or not *Fim-3* and *Evi-1* belong to the same locus. Another approach would be to identify which coding gene is activated by insertion in each locus and to determine whether insertion in the other region results in the activation of the same gene.

A cluster of growth factor and growth factor receptor genes has been found on the long arm of human chromosome 5, including IL3, GM-CSF, PDGF-receptor, c-*FMS*, and M-CSF genes (34). IL3 and GM-CSF have been localized on mouse chromosome 11 (1). Genetic analysis of interspecific

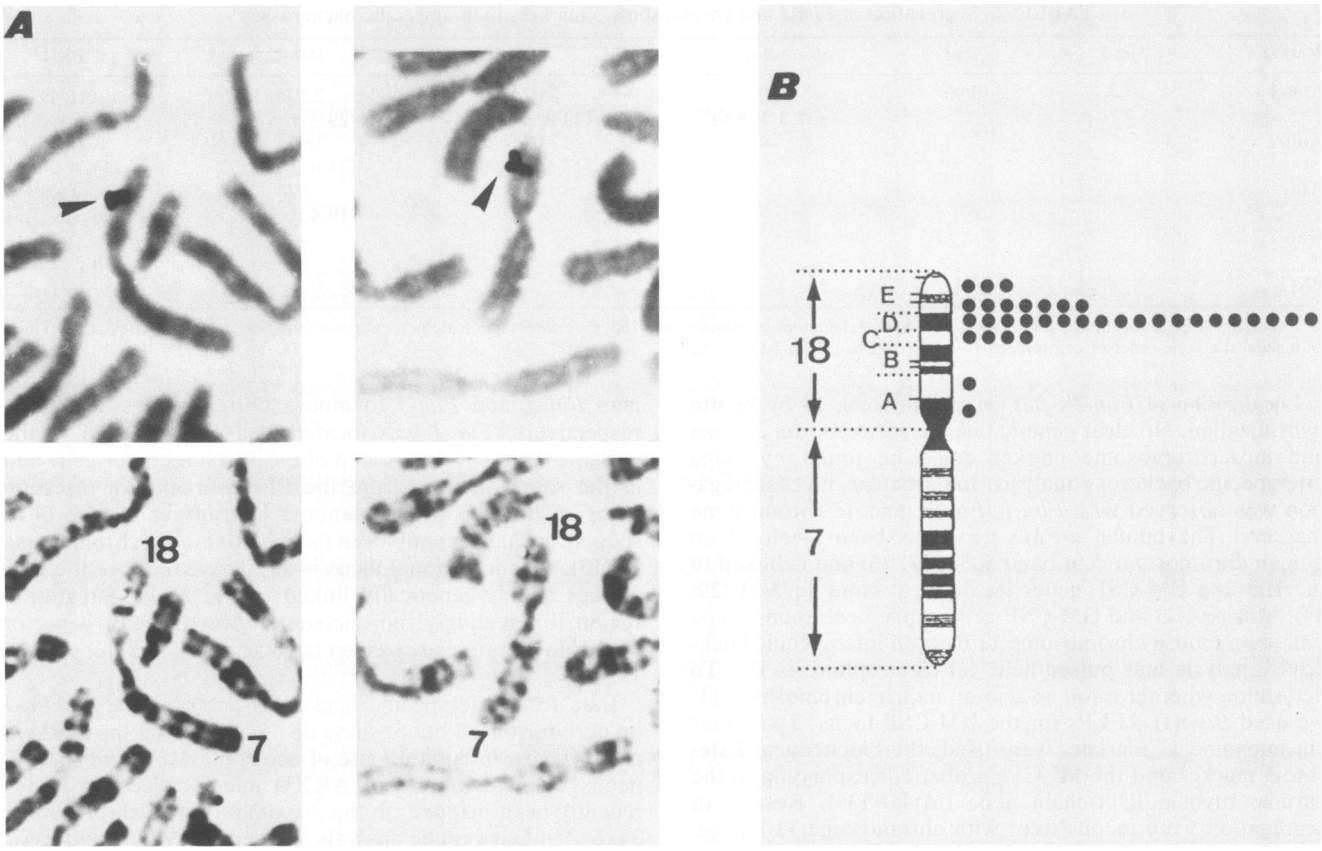

FIG. 1. (A) Two partial WMP mouse metaphases showing the specific site of hybridization to chromosome 18. Top, Arrowheads indicate silver grains on Giemsa-stained chromosome, after autoradiography. Bottom, Chromosomes with silver grains were subsequently identified by R-banding (fluorochrome-photolysis-Giemsa method). (B) Diagram of WMP mouse Rb (7.18) chromosome indicating the distribution of silver grains on 35 ^{3}H-labeled chromosomes 18.

backcross mice did not reveal cosegregation between c-*fms*, GM-CSF, and two other chromosome 11 markers, Esterase-3 and $MLC1_A$. No linkage with any other chromosome being found, we localized c-*fms* by in situ hybridization on the D band of mouse chromosome 18. This confirms the recent localization of c-*fms* on mouse chromosome 18 by Southern analysis of somatic cell hybrid DNAs (17). Location of c-*fms* on mouse chromosome 18 strengthens recent observations on the different organization of the growth factor and receptor genes in humans and mice. Indeed, the M-CSF which is located on human chromosome 5 is not found on mouse chromosome 11 (4), while G-CSF, which is on human chromosome 17q21 (18), is on mouse chromosome 11 (4).

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